**PREPARATION OF COMPETENT *E. COLI* CELLS USING $CaCl_2$**

2006

| **PREPARE SOLUTIONS** | |
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| 1. Luria-Bertani (LB) media (1 L): | Mix **10** g of Bacto-tryptone, **5** of Yeast extract, and 10 **g** of NaCl (for taste). pH to 7.5 w/ NaOH. And $dH_2O$ to 1 L (Autoclave) |
| 2. 1M $CaCl_2$ (1 L): | Mix **111** g of $CaCl_2$ (anhydrous) and **1** L of $dH_2O$. Filter sterilize through a 0.22µ filter |
| 3. 0.1M $CaCl_2$ (1 L): | Mix **100** mL of 1M $CaCl_2$ with **900** mL of $dH_2O$. Filter sterilize through a 0.22µ filter |
| 4. 50% Glycerol (500 mL): | Mix **50** mL of Glycerol with **50** mL of $dH_2O$ (Autoclave) |
| 5. 0.1M $CaCl_2$ + 15% glycerol: | Mix **100** mL of 1M $CaCl_2$, **300** mL of 50% Glycerol, and **600** mL of $dH_2O$ |
| 6. LB plates: | Mix **500** mL of LB media with **7** g of Agar (Autoclave). Cool to ~55-65ºC prior to pouring. The addition of antibiotics should be made before pouring and at a temperature not higher than 55ºC. Antibiotics can also be spread on previously made plates, but this is not very effective (unequal absorption, etc...) |

**PROCEDURE**

1. Streak *E.coli* cells (DH5α, HB101, GM8) on an LB plate; (BL21(DE3)LysS cells on LB plate+34 mg/ml chloramphenicol)

2. Allow cells to grow at 37ºC overnight

3. Place one colony in 10 mL LB media (+antibiotic selection if necessary), grow overnight at 37ºC

4. Take 2 ml LB media and save for blank. Transfer 5 mL overnight DH5α culture into 500 mL LB media in 3 L flask

5. Allow cell to grow at 37ºC (250 rpm), until $OD_{600}$= 0.4 (~2-3 hours)

6. Transfer cells to 2 centrifuge bottles (250 mL), and place cells on ice for 20 mins

7. Centrifuge cells in Sorval GSA rotor at 4ºC for 10 mins at 3,000 g

Subsequent resuspensions may be done in the same bottle. **Cells must remain cold for the rest of the procedure**: Transport tubes on ice and resuspend on ice in the cold room

8. Pour off media and resuspend cells in 30 mL of **cold** 0.1 M $CaCl_2$. Transfer the suspended cells into 50 mL polypropylene falcon tubes, and incubate on ice for 30 mins

9. Centrifuge cells using Sorval RT6000B rotor at 4ºC for 10 mins at 3,000 g (2500 rpm)

10. Pour supernatant and resuspend cells (by pipetting) in 8 mL cold 0.1M $CaCl_2$ containing 15% glycerol. Transfer 140 µL into (1.5 mL) Ependorff tubes placed on ice. Freeze the cells in liquid nitrogen. Cells stored at -80ºC can be used for transformation for up to ~6 months

NOTE: through the process, cells should be treated with care. No vortexing or excess pipetting should be performed, specially when the cells have been resuspended in $CaCl_2$ because lysis will result, decreasing the amount of competent cells). Also, depending on the density of the cells, higher or lower volumes $CaCl_2$ can be used to increase the concentration of cells per tube.

A similar protocol has been described using $MgCl_2$. See Sambrook J. Fritsch E.F. and T. Maniatis (1989). Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press. p.1.74.

Sources of commercial competent cells include: Clontech, Novagen (their NovaBlue Singles are excellent), and Invitrogen (One Shot competent cells)